CHAPTER II
LITERATURE REVIEW

2.1 Lactic acid and its application

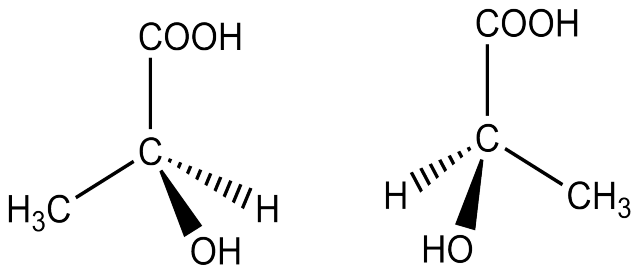
Lactic acid is an organic acid that has been known as milk acid which has a chemical formula as $CH_3CH(OH)COOH$ or $C_3H_6O_3$. It is a colorless to yellowish, odorless hydroscopic crystal or syrupy liquid. Physical and chemical properties of lactic acid are mentioned in Table 2.1 (Dusselier et al., 2013). Lactic acid is a weak acid with pKa 3.86 which partially dissociates in water to release hydrogen ions (eq1) (Ameen and Caruso, 2017).

$$H_3C\text{-}CH(OH)\text{-}COOH \longleftrightarrow H^+ + H_3C\text{-}CH(OH)\text{-}COO^- \qquad \text{(Eq. 1)}$$

Table 2.1 Physical and chemical properties of lactic acid

Lactic acid properties	Details
Compound name	Lactic acid
IUPAC name	2-hydroxypropanoic acid
Appearance	Colorless to yellowish, crystal or syrupy liquid
Solubility	Water, alcohol, ether
Boiling point	122 °C
Melting point	16.8 °C
Molar mass	90.08 g.mol^{-1}
Specific density	1.2
Flash point	113 °C

Lactic acid is composed of a chiral carbon with two terminal carbon atoms consisting of a carboxylic group with a methyl group (Narayanan et al., 2004). It exists in two enantiomeric forms: L-(+)-lactic acid form, also known as (S)-lactic acid and D-(-)-lactic acid, also known as (R)-lactic acid (Ameen and Caruso, 2017).



L-(+)-lactic acid D-(-)-lactic acid

Figure 2.1 Isomer form of L (+) and D-(-)-lactic acid

Lactic acid is recognized as an important precursor in various industries including, food, cosmetics, chemical, and green solvent (Sorensen et al., 2022; Sorensen et al., 2023). While lactic acid is widely used as a food addictive, food preservative, flavoring agent, and scent in food industries, it is also a common ingredient in the cosmetic industry, such as moisturizers, skin-lightening agents, pH regulator, and anti-acne agents. Additionally, in the chemical industry, lactic acid serves various purposes, acting as a descaling agent, pH regulator, neutralizer, chiral intermediate, green solvent, cleaning agent, and slow acid releasing agent. Furthermore, it is an essential chemical feedstock that functions as a precursor to a number of significant chemicals, including propylene oxide, acetaldehyde, acrylic acid, propionic acid, 2,3-pentanedione, ethyl lactate, dilactide, and polylactic acid (Abd Alsaheb et al., 2015). Lactic acid also acts as a precursor to polylactic acid (PLA), a biodegradable polymer widely used in packaging, textiles, and biomedical applications. PLA is eco-friendly and serves as an alternative to petroleum-based plastics, contributing to sustainable practices in the chemical industry (Wu et al., 2023a).

2.2 Lactic acid production

Lactic acid can be produced by chemical synthesis with different pathways and microbial fermentation from renewable resources (Wee et al., 2006). Lactic acid (racemic mixture) can be synthesized by hydrocyanation, followed by cyanohydrin hydrolysis and generating ammonium sulfate ($(NH_4)_2SO_4$) as a byproduct (Figure 2.2). However, hydrogen cyanide (HCN) is known as hazardous chemical, it is challenging for

handling HCN and complicated purification processes are required to obtain food-grade lactic acid. Additionally, in sectors where purity and environmental concerns are considered as crucial, fermentation-based methods are often preferred due to their natural origin and environmentally friendly characteristics. Lactic acid is also produced through the fermentation of carbohydrates or fermentable sugars, typically sugars such as glucose or lactose, by lactic acid bacteria. Lactic acid bacteria (LABs), such as *Lactobacillus* species, naturally produce lactic acid during fermentation (Hoyos et al., 2017).

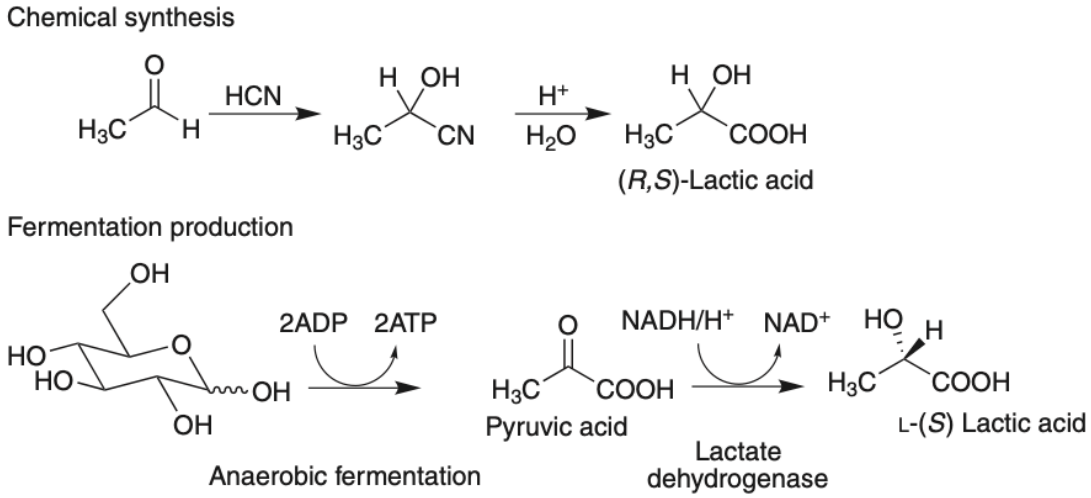


Figure 2.2 Lactic acid production (Hoyos et al., 2017)

2.3 Microbial fermentation of lactic acid

Lactic acid can be produced by various microorganisms, mostly by lactic acid bacteria (LAB). Generally, LAB belong to gram positive bacteria, non-forming spore, and anaerobic fermenting bacteria such as *Lactobacillus*, *Bacillus, Enterococcus, Vagococcus, Leuconostoc*, *Streptococcus, and Pediococcus* (Nuraida, 2015; Panagiota et al., 2013). Fungi strains such as *Rhizopus oryzae* could produce L-(+)-lactic acid from starch and glucose (Zhou et al., 1999) while bacteria strains could produce both L-(+)-lactic acid and D-(-)-lactic acid or a mixture of DL-lactic acid (Zhao et al., 2010). The underlying of metabolic pathways of lactic acid production are shown in Figure 2.3 in which 2 molecules of lactic acid are produced from glucose under homofermentative pathway whereas lactic acid and by-products such as ethanol and carbon dioxide (CO_2)

are produced under heterofermentative pathways (Mora-Villalobos et al., 2020). Currently, bacterial strains have remained the preferred choice among researchers for lactate production due to their capability to produce lactic acid at high yield and productivity (Oonkhanond et al., 2017). Table 2.2 shows some examples of D-(-)-lactic acid and L-(+)-lactic acid biotechnological production. Similarly, microorganisms that are targeted for industrial lactic acid production must possess key attributes. These include enhancing productivity to reduce time, improving yield to lower substrate costs, utilizing low-cost media or substrates to achieve high concentrations, minimizing by-product formation to enhance purification efficiency, and demonstrating resilience against contamination and infections (Auras et al., 2010).

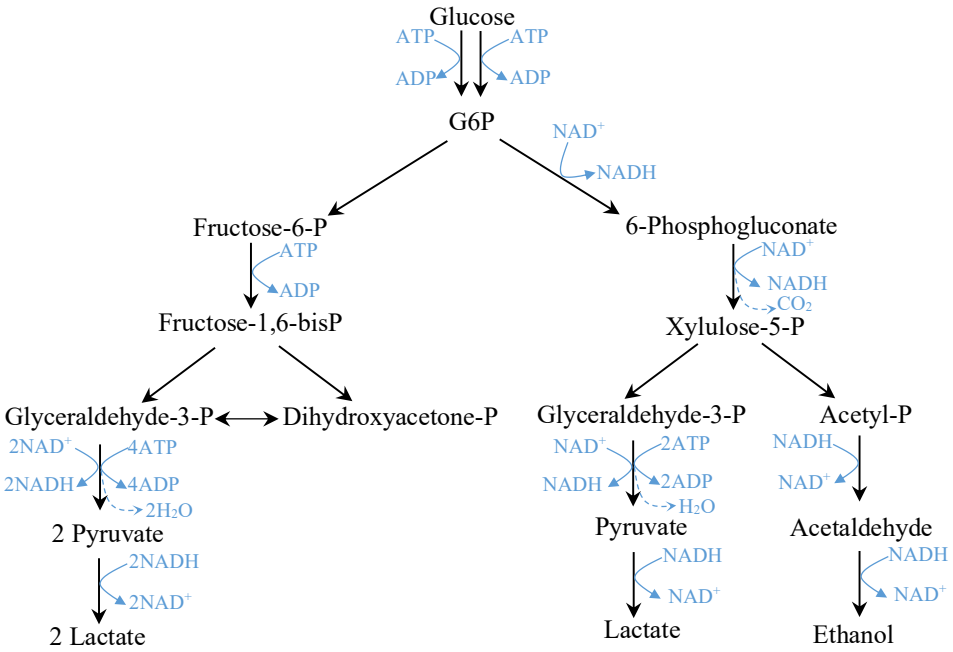


Figure 2.3 Metabolic pathways of lactic acid production by lactic acid bacteria

Table 2.2 D(-) and L-(+)-lactic acid production by microorganisms

Types of lactic acid	Microorganism	Substrate	Fermentation	Lactic acid titer (g/L)	Yield (g/g)	Productivity (g/L/h)	References
L (+)	*B. coagulans* NCIM 5648	Sugarcane bagasse	SHF	69.2	0.76	2.88	(Baral et al., 2020)
D (-)	*L. delbrueckii* subsp. *bulgaricus*	Beechwood	SSF	62	0.69	0.86	(Karnaouri et al., 2020)
D (-)		Pine	SSF	36.4	0.40	1.6	
D (-)	*P.acidilactici ZY15*	Wheat straw	SSCF	128.1	0.69	1.78	(He et al., 2023)
L (+)	*E.mundtii WX1* and *L.rhamnosus*	Corn stover	SHF	34.6	0.9	1.73	(Klongklaew et al., 2023)
L (+)	*E. mundtii* CGMCC 22227	Garden garbage	SHF	59.7	0.62	0.79	(Zhu et al., 2023)

SHF: separate hydrolysis and fermentation, SSF: simultaneous saccharification and fermentation, SSCF: simultaneous saccharification and co-fermentation

2.4 Fermentation modes

The microbial fermentations are carried out in various strategies which plays a crucial role in determining the efficiency, productivity, and yield of the desired fermentation products. The most common fermentation modes are shown in Figure 2.4: batch, fed-batch, and continuous fermentation (Kacaribu and Darwin, 2024). Table 2.3 represents advantages and disadvantages of each process.

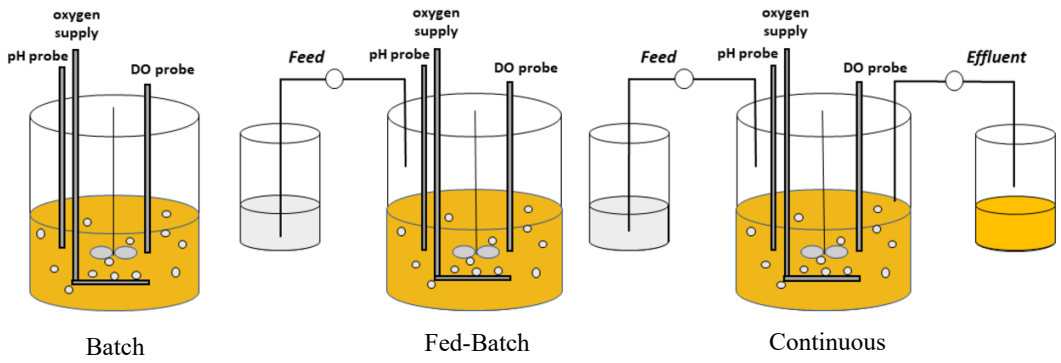


Figure 2.4 The modes of fermentation: batch, continuous, and fed-batch processes (Rajpurohit and Eiteman, 2022)

2.4.1 Batch fermentation

Batch fermentation is simpler and a closed system where all the nutrients, microorganisms, and substrates are all added at the beginning of process, therefore, only acid or alkaline is added during fermentation to control the pH. Likewise, the microorganisms grow through several phases, including the lag phase, exponential or log phase, stationary phase and death phase (Figure 2.5). The desired products are harvested when the operation is completed. Although this mode of fermentation shows lower productivity and inhibition by substrate or product accumulation, batch fermentation is widely used as it minimizes the contamination risk and can be operated easily (Rawoof et al., 2021).

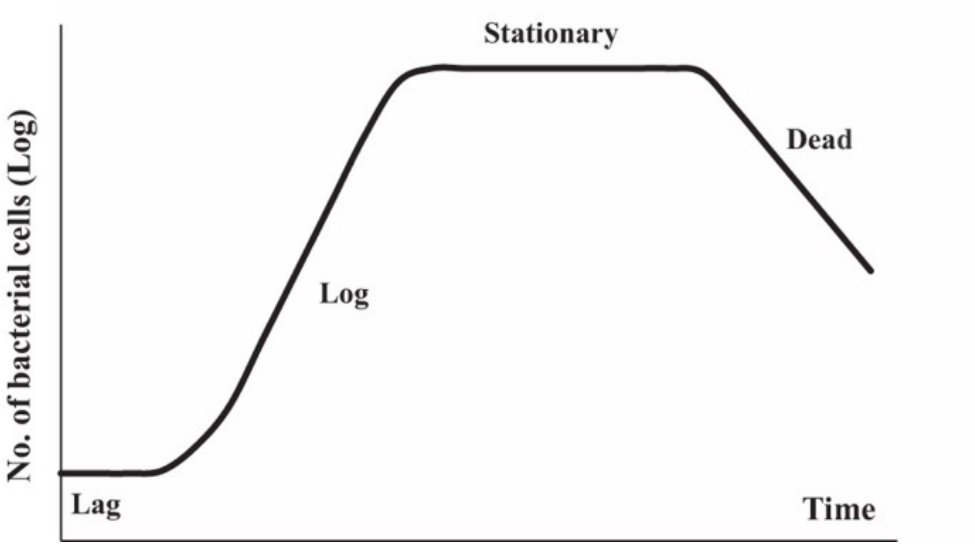


Figure 2.5 The growth curve of bacterial in culture medium (Wang et al., 2015)

2.4.2 Fed-batch fermentation

Fed-batch strategy is conducted to avoid substrate inhibition and prolonged productive phase of microorganism; hence, increment in product titer (Behl et al., 2023). The substrates are added into the fermenter during the operation continuously or in pulses over a period of time without removing the fermentation broth (Mohamed et al., 2021). This method is especially beneficial when substrate can inhibit cell growth or product formation at high concentrations; subsequently, it provides higher productivity and reduces operation times (Tang et al., 2010).

2.4.3 Continuous fermentation

Continuous fermentation is usually performed to extend the exponential phase and enhance productivity without increasing the broth volume. To do so, the fresh medium was continuously added into the fermenter tank while the cell broth is simultaneously removed from the bioreactor at the optimized flow rate and time (Sen and Roychoudhury, 2013). This method is often used in a large scale or industry, while it is not broadly used in laboratories (Mohamed et al., 2021).

Table 2.3 The advantages and disadvantages of each fermentation modes: batch, fed-batch, and continuous processes. Adapt from (Yang et al., 2024)

Fermentation modes	Advantages	Disadvantages
Batch	• Simple to operate • Low risk of contamination and strain mutation	• Relatively slow substrate utilization • Growth of microbes is limited by the nutrient concentration in the medium
Fed-batch	• High cell densities • Minimizes the inhibition of high concentrations of substrate and end-products.	• Longer duration • Slow response of cells to the changes in pH
Continuous	• High productivity and effectiveness • Less sterilization and re-inoculation • Easy to control automatically • Cells can be harvested throughout • Avoids the inhibition caused by a mass of substrate and the cells • Less maintenance cost	• Long-term fermentation may lead to blockages • Risk of contamination

2.5 *K. oxytoca* KIS004-91T

Klebsiella oxytoca is a gram-negative bacterium that is non-motile and rod-shaped in the family *Enterobacteriaceae*. It can be isolated from various tissues of clinically affected individuals and animals, as well as from the skin, mucous membranes, oropharynx, and intestines of both healthy humans and animals (Darby et al., 2014). *K. oxytoca* is extensively utilized on an industrial scale for producing biofuels and biochemicals because of its remarkable ability to efficiently utilize various substrates. *K. oxytoca* KIS004-91T strain was previously engineered to enhance D-(-)-lactic acid production via fermentation in a low nutrient medium. Initially, Sangproo et al., (2012) deleted the *adhE* (alcohol dehydrogenase) gene, responsible for ethanol synthesis, in the *K. oxytoca* M5a1 strain, resulting as the KMS002 strain (Δ*adhE*). Subsequently, the *pta-ackA* (phosphor transacetylase-acetate kinase A) genes, involved in acetyl-CoA and acetate production were deleted, yielding the strain KMS004 (Δ*adhE,* Δ*pta-ackA*). These modifications led to increased D-(-)-lactic acid production rates when using glucose and sugarcane molasses as substrates. Ethanol was not produced while other by-products such as succinate, formate, acetate, and butanediol were still detected in the fermentation broth using KMS002 (Δ*adhE*) and KMS004 (Δ*adhE,* Δ*pta-ackA*) strains. Even though the acetate kinase gene was removed in KMS004 (Δ*adhE,* Δ*pta-ackA*), formate and acetate were still the major by-products formation, likely due to the presence of the *pflB* (pyruvate-formate lyase) gene, which is responsible for converting pyruvate to acetyl-CoA and formate. Consequently, acetyl-CoA may be converted to acetate by alternate acetate kinase isoenzymes. In the anaerobic pathway, *K. oxytoca* KMS004 strain exhibited higher D-(-)-lactic acid titers and yields compared to the aerobic pathway, with reduced by-product formation. Under anaerobic conditions, glucose is converted to pyruvate, which can then be converted to D-(-)-lactic acid via lactate dehydrogenase A by using NADH. Alternatively, pyruvate can be converted to acetyl-CoA in which the wild type strain, can be further converted to ethanol and acetate. However, acetyl-CoA is solely converted to acetate due to deletions of *adhE* and *pta-ackA* genes in KMS004 strain. Additionally, fumarate

reductase plays a crucial role in the anaerobic metabolism of various microorganisms. This enzyme is responsible for catalyzing the reduction of fumarate to succinate, with fumarate acting as the terminal electron acceptor. The *frd* gene, encoding fumarate reductase, is mainly found in microorganisms such as *Escherichia coli* and *Klebsiella oxytoca*. Therefore, three main pathways for NADH oxidation are described, which involve the production of lactate, succinate, and ethanol via lactate dehydrogenase, fumarate reductase, and alcohol dehydrogenase, respectively; in the mixed-acid pathway (Zhang et al., 2009). Therefore, eliminating the *frd* gene could enable direct NADH oxidation towards lactic acid production rather than succinic acid production in the KMS004 strain. Thus, further enhancing D-(-)-lactic acid production in KMS004 strain by eliminating the *pflB* gene, responsible for formate and acetyl-CoA production, could potentially improve D-lactic acid yield by reducing the formation of other by-products. KMS004 (Δ*adhE,* Δ*pta-ackA*) strain was then further deleted *frd* and *pflB* gene to eliminate succinate-producing pathway and to decrease other by-product formation such as acetate and 2,3-butanediol, yielding KIS002 (Δ*adhE,* Δ*pta-ackA,* Δ*frd*) and KIS004 (Δ*adhE,* Δ*pta-ackA,* Δ*frd,* Δ*pflB*) strains, respectively. The fermentation pathway of *K. oxytoca* KIS004 strain are shown in Figure 2.6 (In et al., 2020). Under anaerobic conditions, KIS002 produced 35.4 g/l of D-(-)-lactic acid with yield of 0.75 g/g and productivity of 0.59 g/l/h without succinate, in AM1 medium containing 5% (w/v) glucose. Other by-products such as acetate and 2,3-butanediol, were still produced by KIS002 at concentrations 0.865 g/L and 0.12 g/L, respectively. After deletion of *pflB* gene, 45.2 g/L of D-(-)-lactic acid were produced by KIS004 in AM1 medium containing 5% (w/v) glucose under anaerobic conditions with a yield of 0.96 g/g and productivity of 0.47 g/L/h. Hence, acetate was not produced with KIS004 while lower level of 2,3-butanediol was found compared to KMS004 strain. In order to improve glucose utilization, metabolic evolution (Jantama et al., 2008), was conducted on the KIS004 strain. The KIS004 strain was cultured in LB medium containing 2% (w/v) glucose and transferred to a newly fresh AM1 medium containing 10% (w/v) glucose every 24 h until the mutant strain showed no further improvement. As KMS004 no longer

produces acetate due to the removal of pyruvate formate lyase, it has become auxotrophic for acetate. After the 18th transferred, metabolic evolution was conducted by supplementing 20 mM sodium acetate into the medium. The acetate concentration was gradually decreased along with the growth rate of strain until the strain grew efficiently without the need for external acetate supplementation. Consequently, glucose was completely utilized after the 91th transferred and 98.6 g/L of D-(-)-lactic acid were produced without any by-product formations, resulting as KIS004-91T strain. D-(-)-lactic acid was produced using *K. oxytoca* KIS004-91T strain at 101 g/L and 126 g/L from 10% glucose (w/v) in batch and fed-batch fermentations using glucose as a substrate, and was achieved 98.4 g/L from cassava starch under SHF condition (In et al., 2020). Examples of D-(-)-lactic acid production using *K. oxytoca* are shown in Table 2.4.

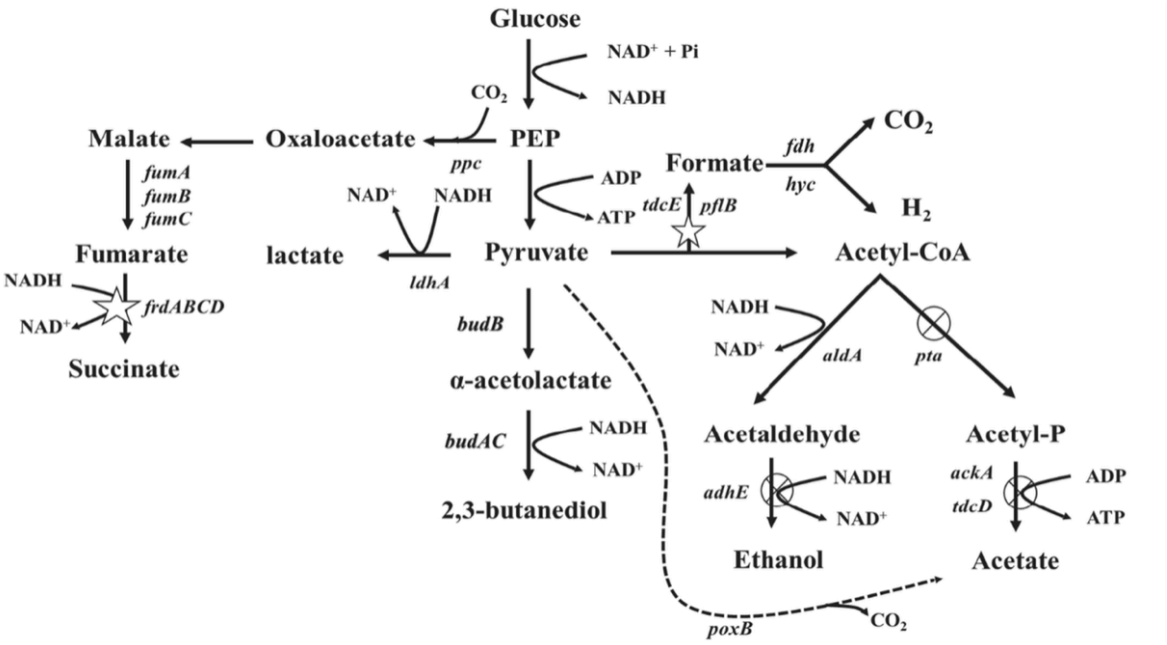


Figure 2.6 Metabolic pathway of *K. oxytoca* strain under anaerobic conditions (In et al., 2020). Solid arrows represent central fermentative pathways. Dot arrow represents an alternative acetate-producing pathway via *poxB*. Cross signs represent gene deletions previously performed in *K. oxytoca* KMS004 (Δ*adhE*Δ*ackA-pta*) strain. Star signs represent deletions of *frd* and *pflB* genes in *K. oxytoca* KIS004 (Δ*adhE*Δ*ackA-pta*Δ*frd*Δ*pflB*) strain.

Table 2.4 D-(-)-lactate production by *K. oxytoca* strain

Types of lactic acid	Microorganism	Substrate	Fermentation	Lactic acid titer (g/L)	Yield (g/g)	Productivity (g/L/h)	References
D-(-)	*K. oxytoca* KMS002	Maltodextrin	SHF	33.6	0.92	0.35	(Sangproo et al., 2012)
D-(-)	*K. oxytoca* KMS004	Maltodextrin	SHF	32.9	0.91	0.34	
D-(-)	*K. oxytoca* KIS004-91T	Sugarcane bagasse	Fed-batch SHF	101.0	0.99	1.94	(Gosalawit et al., 2024)
D-(-)	*K. oxytoca* KIS004-91T	Cassava starch	SHF	98.4	0.93	1.24	(In et al., 2020)

SHF: separate hydrolysis and fermentation

2.6 Lignocellulose biomass

Lignocellulose is an abundant and renewable source for biochemical fermentation due to its composition–cellulose and hemicellulose, about 70% (w/w), were observed. Thus, lignin links cellulose and hemicellulose with covalent and hydrogen bonds. Typically, cellulose is comprised of glucose monomers, which is condensed via ß-(1,4)-glycosidic bonds, forming microfibrils with hydrogen bonds, and linear chains of unbranched polymers. The hydrogen bonds and van der Waals forces in cellulose result in a highly crystalline and rigid structure of lignocellulose (Davison et al., 2013; Gavila et al., 2015). In contrast, hemicellulose is known as short-branched polymers and consists of pentose and hexose, including xylose, arabinose, glucose, mannose, and galactose, respectively. Thus, some uronic acids (acetyl, glucuronic acid, and arabinose side groups) were found in hemicellulose too (Kumar et al., 2020). Unlike cellulose, the crystallinity of hemicellulose is low and more easily hydrolyzed; whereas, it can be removed by sodium hydroxide or precipitated by acidic alcohol (Betts et al., 1991). Otherwise, lignin consists of aromatic polymers such as p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, forming p-hrdorxyphenyl (H), guaiacyl (G), and syringyl (S) units, respectively, generating highly branched and cross-linked polymers (Borrero-Lopez et al., 2022). The compositions of these units depend on the type of plants; for instance, hardwoods are mostly built with S and G units, softwoods mainly consist of G units, whereas grasses contain all H, G, and S units (Yankov, 2022). Cellulose and hemicellulose were packed together by hydrogen bonds; thus, lignin cross-linking between these two components via covalent bonds results in a recalcitrant structure and resistance of lignocellulose materials (Ufodike et al., 2020; Vasic et al., 2021). Table 2.5 shows the composition of different types of lignocellulose biomass, including cellulose, hemicellulose, and lignin contents.

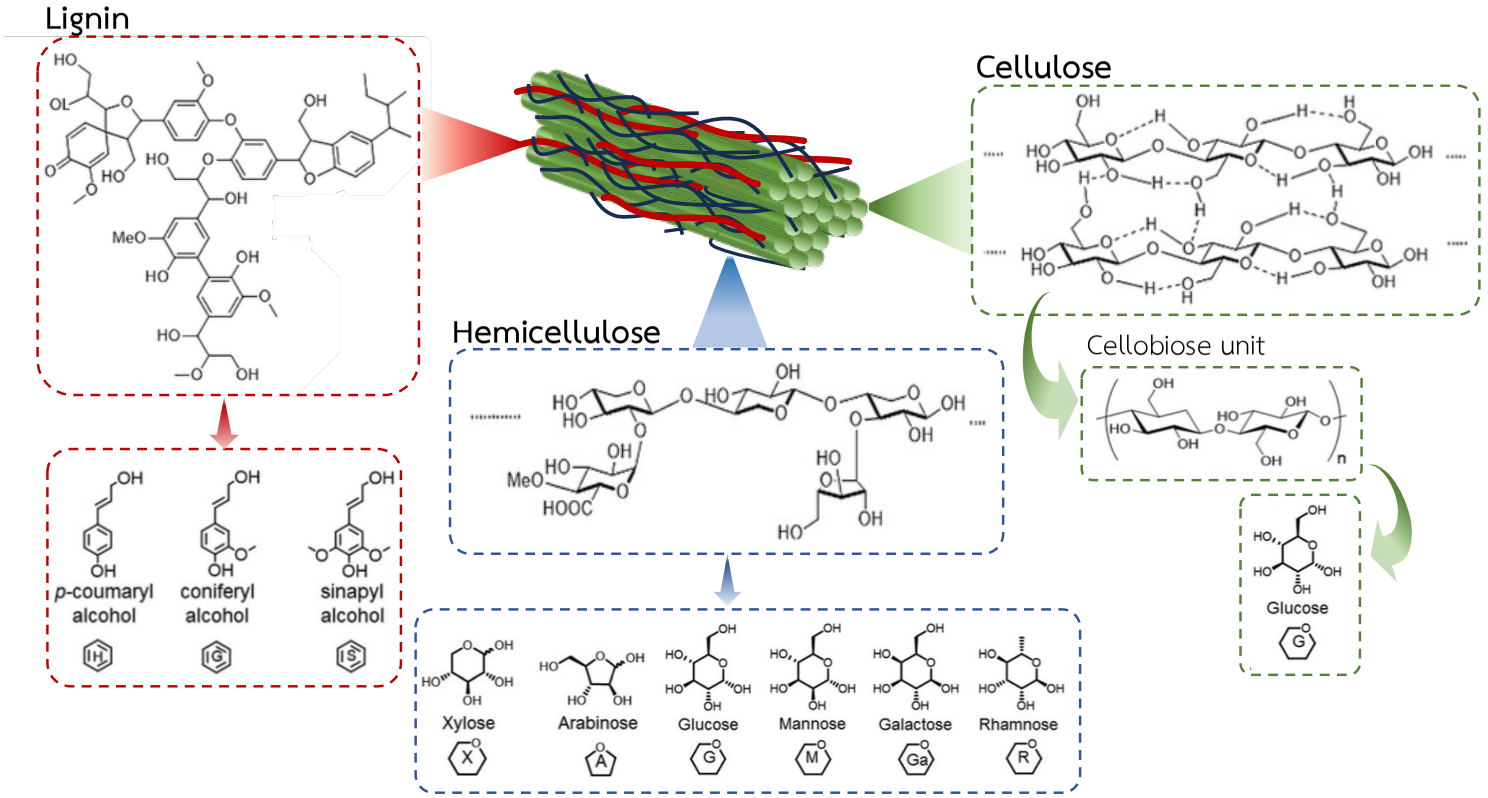


Figure 2.7 Lignocellulose structure and composition including cellulose, hemicellulose, and lignin. Adapted from (Isikgor and Becer, 2015)

Table 2.5 Different types of lignocellulose biomass and its composition

Type of lignocellulosic	Cellulose	Hemicellulose	Lignin	References
Pine sawdust	24.9	31.5	36.6	(Rapado et al., 2021)
Cassava bagasse	13.5	5.8	2.8	(Chen et al., 2020)
Rice husk	47.6	19.1	19.3	(Jaichakan et al., 2021)
Soybean hulls	35.8	23.1	9.1	(Rojas et al., 2014)
Wheat straw	41.1	37.5	13.5	(Grewal and Khare, 2018)
Barley hull	34	36	19	(Kim et al., 2008)
Cotton stover	38	23	20	(Wan and Li, 2010)
Napier grass	47	31	22	(Reddy et al., 2018)
Waste papers	65	13	1	(Chen et al., 2004)
Rice straw	38	32	12	(Lu and Hsieh, 2012)
Corn cob	41	31	12	(Chen et al., 2010)
Brewers spent grains	21.7	19.3	19.4	(Meneses et al., 2013)
Wheat	18.6	34.1	9.5	(Mikulski and Klosowski, 2018)
Pineapple leaves	72.76	17.15	4.76	(Nashiruddin et al., 2020)
Pineapple leaves	66.2	19.5	4.2	(Daud et al., 2014)

2.7 Pretreatment of lignocellulosic biomass

The complex network and resistance of lignocellulosic biomass was naturally attributed to prevent the accessibility of pathogens and enzymes. Therefore, pretreatment was performed to reduce the crystallinity and polymerization degree by breaking the covalent and hydrogen linkages of cellulose, hemicellulose, and lignin,

hence increasing the accessible surface area for further fermentable sugars conversion via enzymatic hydrolysis (Kumar et al., 2009; Vasic et al., 2021). Different methods of pretreatment, including physical, chemical, physiochemical, and biological, were employed to lignocellulose biomass for hemicellulose and lignin removal, thus limiting the degradation of cellulose (Jonsson and Martin, 2016). Generally, physical pretreatment such as chopping, grinding, and milling was conducted to reduce the size of biomass to the particle size as well as the crystallinity, improving digestibility of enzymes (Millett et al., 1976). Meanwhile, chemical pretreatment, including acid, alkaline, oxidative, and organic solvent, was differently applied to enhance enzyme accessibility by removing lignin or dissolving hemicellulose, thus disrupting cellulose structure. Acid pretreatments of lignocellulose are employed for hemicellulose and lignin solubilization, in which either concentrated or diluted acid can be used for acid hydrolysis (Sun and Cheng, 2002). Concentrated acid hydrolysis (30-70%) can be done at low temperature with normal atmosphere, whereas diluted acid hydrolysis (0.1-2%) requires high temperature and pressure to solubilize hemicellulose and lignin (Den et al., 2018). Using acid (HCl, H_2SO_4, H_3PO_4, and HNO_3) for pretreating biomass triggers inhibitors formation (furfural, HMF, and acetic acid) which require subsequent detoxification step (Himmel et al., 1997; Saha et al., 2005). Hence, safety equipment is required during acids pretreatment due to its corrosive and toxicity nature. Unlike acid hydrolysis, alkaline pretreatment using sodium, potassium, calcium, and ammonium are mainly target lignin, breaking ester linkages and disrupting the lignin-carbohydrate bonds (LCC) with partially dissolve hemicellulose while cellulose are remained intact. The solubilization of lignin and hemicellulose and swelling of cellulose offers larger interact surface area for subsequent enzymatic hydrolysis, increasing fermentable sugar formation (Behera et al., 2014). Maurya et al. (2015) mentioned alkaline pretreatment increases accessibility of enzyme to the cellulose due to the removal of acetyl group and uronic acid substitutions in hemicellulose during pretreatment, thus, NaOH was reported as the most effective base among all bases for pretreating lignocellulose (Kim et al., 2016). On the other hand, oxidative pretreatment such as addition of hydrogen

peroxide (H_2O_2) and peracetic acid ($C_2H_4O_3$) remove lignin and hemicellulose from biomass (Garcia-Cubero et al., 2009). Within this pretreatment, alkyl/ aryl ether bonds were cleaved off, disruption of side chained and lignocellulose structure (Maurya et al., 2015; Patel et al., 2023). Similarly, organosolv process could significantly remove lignin and hemicellulose while cellulose remained untouched (Sun and Chen, 2008). Organic solvents such as methanol, ethanol, acetone, and ethylene/ tri-ethylene glycol can be used in this method. Therefore, a proper removal of organic solvent after pretreated is necessary since most of them could inhibit enzyme activity during enzymatic hydrolysis (Mosier et al., 2005). Likewise, physiochemical methods including steam explosion, liquid hot water (LHW), and ammonia-based pretreatment (AFEX), improves enzyme digestibility via combination of physical forces and chemical reactions. These methods promote hemicellulose degradation and lignin disruption (Pan et al., 2005) in which inhibitors are formed accordingly, limiting the enzymatic hydrolysis and fermentation process (Oliva et al., 2003). Normally, biological pretreatment is employed for delignification and hemicellulose degradation using microorganism (mainly fungi). Even though, this method offers lower production cost, the lower hydrolysis rate with longer processing time is considered as disadvantages compared to other methods (Sanchez, 2009; Shi et al., 2008; Sun and Cheng, 2002). The advantages and disadvantages of different methods of pretreatment are shown in Table 2.6.

Table 2.6 The advantages and disadvantages of different methods of pretreatment. Adapted from (Maurya et al., 2015)

Pretreatment method	Advantages	Disadvantages
Milling/Grinding	• Decrease crystallization and degree of polymerization of cellulose • Increase surface area and pore size of biomass	• High power and energy consumption
Alkaline	• Lignin removal • Low inhibitor formation • Decrease cellulose crystallization	• High cost of alkaline catalyst
Concentrated acid	• High glucose yield • Ambient temperature	• High cost of acids catalyst • Corrosion-resistant equipments are required • Inhibitor formation
Diluted acid	• High sugars recovery • Hemicellulose solubilization • Low formation of toxic products	• Inhibitor formation • Detoxification step is required • Need high temperature and pressure

Table 2.6 The advantages and disadvantages of different methods of pretreatment. (continued)

Organosolv	• Hydrolysis of lignin and hemicellulose	• High cost • Solvent recovery and recycling are complicated
Steam explosion	• Cost effective • Hemicellulose solubilization and lignin transformation\ • High yield of glucose and hemicellulose in two-step method	• Generation of toxic compounds • Partial degradation of hemicellulose
Liquid hot water (LHW)	• No chemicals are required • Hemicellulose solubilization	• High energy and water are required • Formation of toxic compound
Ammonia fiber expansion (AFEX)	• Increase surface area • Less inhibitor formation	• Inefficiency of lignin removal • High cost of ammonia • Ammonia recycling is required

Table 2.6 The advantages and disadvantages of different methods of pretreatment. (continued)

Biological	• Cost effective • Delignification • Partial hydrolysis of hemicelluloses • No chemical requirements • Reduction in degree of polymerization of cellulose • Mild environmental condition	• Require amount of time for pretreatment • Lower productivity

2.8 Enzymatic hydrolysis

Enzymatic hydrolysis is a subsequent crucial step after pretreatment process to breakdown polysaccharide of lignocellulose biomass into fermentable sugars to be readily for fermentation step. Generally, this action is carried out under mild conditions in which the optimum temperature ranges between 40-50 °C and pH of 4.5-5.0 (Vasic et al., 2021). During hydrolysis, either cellulose or hemicellulose are cleaved off into simpler sugars such as glucose, cellobiose, and cello-oligosaccharides or xylose, mannose, fructose, and arabinose (Choi et al., 2020; Linton, 2020; Singh et al., 2003). Cellulases play an important role in depolymerization the crystallinity of cellulose by which endoglucanase initiate the hydrolysis of cellulose, randomly split the chains of amorphous region of cellulose by hydrolyzing ß-1,4-glucosidic bond. Meanwhile, exoglucanases cut down the chains of cellulose from the reducing and non-reducing ends of cellulose chains, producing cellobiose and glucose subunits. Then ß-glucosidase or cellobiase breaks cellobiose into two glucose molecules (Mohamed et al., 2011; A. Patel et al., 2019; Srivastava et al., 2018). Figure 2.8 shows the action mode of cellulase enzymes breakdown cellulose.

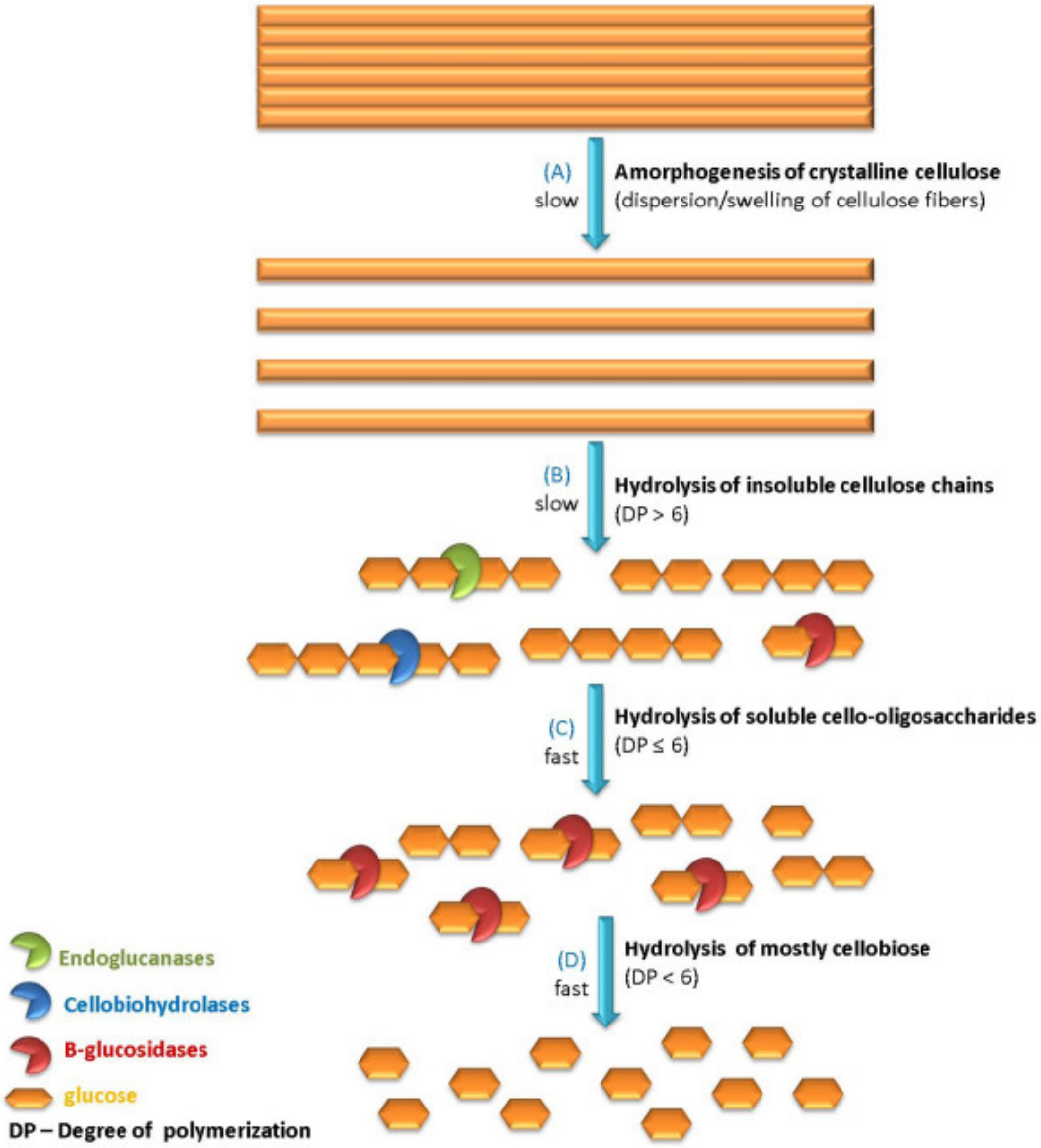


Figure 2.8 The hydrolysis of lignocellulose using cellulase enzymes (Arantes and Saddler, 2010)

2.9 Pineapple crown

Pineapple stands as a leading fruit globally, with Costa Rica, Philippines, Brazil, Thailand, and India serving as major producers. In recent years, the demand for pineapple and its derivatives has surged significantly. Pineapple waste increases not only from the consumption of fresh fruit, commercial juice, but also processed items like canned and frozen products during harvesting and processing methods (Mohd Ali

et al., 2020; Roda and Lambri, 2019). Pineapple crown, the leafy top of pineapple fruits, is considered as a major by-product originating from pineapple processing facilities since its weight around 10–25 percent of the fruit's total weight (Prado and Spinace, 2019). Pineapple crowns are an ideal substrate for lactic acid production owing to their abundance, low cost, and rich carbohydrate content, primarily consisting of glucose and fructose. It contains 79-83%, 19%, 5-15% and 1% of cellulose, hemicellulose, lignin and pectin, respectively (Choquecahua et al., 2020). As waste from pineapple processing industries, they offer a readily available and cost-effective source for fermentation processes. Additionally, pineapple crowns contain essential nutrients and minerals that support the growth and metabolism of lactic acid bacteria, promoting efficient fermentation and higher yields of lactic acid (Byresh et al., 2023). By purposing pineapple crowns for lactic acid production, environmental sustainability is also enhanced, as it helps divert organic waste from landfills, aligning with efforts to minimize resource wastage and promote sustainable practices in industrial processes.